

## Regulation of IL-2 $\beta$ receptor expression and $\beta$ -chain mRNA by human thymocytes

G. H. REEM, X. HAN & A. MARCELLI *Department of Pharmacology and the Kaplan Cancer Center, New York University Medical Center, New York, USA*

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### SUMMARY

The high affinity form of the human IL-2 receptor (IL-2R) has two known components, the IL-2R $\alpha$  (p55) and the IL-2R $\beta$  chain (p75). We have previously shown that recombinant IL-2 (rIL-2) could induce the expression of the  $\alpha$ -chain (p55) on T cells and thymocytes, and increase this expression following suboptimal activation with concanavalin A (Con A) in combination with IL-2. An increase in the accumulation of IL-2R $\alpha$ -specific mRNA induced by rIL-2 in T cells and thymocytes had also been documented. We report here that the expression of IL-2R $\beta$  on the cell surface can be demonstrated on human thymocytes by the binding of Mik  $\beta_1$ , a MoAb directed against an epitope of the  $\beta$ -chain. The IL-2R $\beta$  chain is constitutively expressed on freshly isolated thymocytes; this expression can be increased in thymocytes activated with Con A in combination with IL-2 or tetradecanoylphorbol 13-acetate (TPA). Blocking the formation of high affinity receptors with a MoAb directed against the  $\alpha$ -chain of the receptor results in an increase in the display of IL-2R $\beta$  as evidenced by binding of MoAb Mik  $\beta_1$ . The accumulation of IL-2R $\beta$ -specific mRNA is observed in freshly isolated thymocytes and it is increased in thymocytes cultured with rIL-2 alone, with Con A, and further enhanced by the addition of rIL-2 in combination with Con A or with TPA. Cyclosporine (CsA), which inhibits the accumulation of lymphokine-specific mRNA of thymocytes, does not inhibit the induction of the accumulation of IL-2R $\beta$ -specific mRNA. This is analogous to its effect on the expression of the  $\alpha$ -chain (p55), and the accumulation of  $\alpha$ -chain-specific mRNA.

**Keywords** interleukin-2 receptor human thymocytes IL-2R $\beta$   $\beta$ -chain mRNA expression

### INTRODUCTION

Recently  $\beta$ -chain-specific antibodies have been developed [1,2] and the IL-2 receptor  $\beta$  (IL-2R $\beta$ ) chain gene has been cloned and characterized [3]. The existence of an IL-2 binding molecule distinct from the  $\alpha$ -chain (p55) protein has been demonstrated earlier [4,5] and the proliferative response of human thymocytes to recombinant IL-2 (rIL-2), which do not express IL-2R $\alpha$  on the cell surface unless activated, has been observed [6,7].

Expression of IL-2R $\beta$  has been documented by the binding of  $^{125}\text{I}$  IL-2 to leukaemic cells from various haematopoietic lineages and on thymocytes [8–10]. More recently, expression of IL-2R $\beta$  has been demonstrated on a subset of circulating T cells and on a subset of thymocytes [7,9].

The  $\beta$ -subunit of IL-2R binds IL-2 with intermediate affinity; high affinity binding requires the expression of both  $\alpha$ - and  $\beta$ -subunits [11–13]. It has been reported that the  $\beta$ -subunit is essential for signal transduction and that a specific region of the

IL-2R $\beta$  chain is essential for signal transduction and growth, but is not essential for ligand binding and internalization [3,14]. The binding of IL-2 with intermediate affinity may require a putative  $\gamma$ -chain specifically present on lymphoid cells [14–16].

Since in human thymocytes both the expression of the  $\alpha$ -chain and the accumulation of  $\alpha$ -chain-specific mRNA can be induced and increased by rIL-2 [17–20], we studied the modulation of the expression of the  $\beta$ -chain by indirect immunofluorescence with the MoAb Mik  $\beta_1$ , which recognizes the IL-2 binding site on the  $\beta$ -chain [12]; and determined the accumulation of  $\beta$ -chain-specific mRNA with the appropriate probe. We provide evidence that IL-2R $\beta$  is constitutively expressed on human thymocytes, and that this expression can be increased by concanavalin A (Con A) in combination with IL-2 or tetradecanoylphorbol 13-acetate (TPA), and by blocking the formation of high affinity receptors with 2A3, a MoAb directed against the  $\alpha$ -chain. Similarly, IL-2R $\beta$  chain-specific mRNA is expressed in freshly isolated human thymocytes and increased by activation with rIL-2, Con A or TPA, and further enhanced by rIL-2 in combination with Con A or TPA.

In addition, we investigated the effect of cyclosporine (CsA) and found that in analogy with its effect on IL-2R $\alpha$ , CsA does

Correspondence: Dr Gabrielle H. Reem, Department of Pharmacology, New York University Medical Center, New York, NY 10016, USA.

not decrease the expression of IL-2R $\beta$  on the cell surface nor does it inhibit the accumulation of  $\beta$ -chain mRNA induced in activated thymocytes.

## MATERIALS AND METHODS

### Monoclonal antibodies

Monoclonal antibody 2A3 (anti-IL-2R $\alpha$ , mouse IgG1a) was provided by Dr D. Urdal (Immunex Corporation, Seattle, WA). Mik  $\beta_1$  (mouse IgG2a,  $\beta$ -chain MoAb) was a gift from Dr M. Tsudo (the Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan); anti-mouse IgG2a-FITC was purchased from ICN Biomedicals (Lisle, IL), mouse IgG2a-FITC from Becton Dickinson (Mountainview, CA).

### Chemicals

Purified rIL-2 was a gift from Hoffman La Roche (Nutley, NJ) and CsA from Dr J. F. Borel (Sandoz, Basel, Switzerland). Con A was purchased from Sigma Biochemical (St Louis, MO) and TPA from L.C. Service Corporation (Woburn, MA).

### Probes

IL-2R cDNA ( $\alpha$ -chain) was provided by Dr W. C. Greene (Duke Medical Center, Durham, NC), IL-2R $\beta$  chain cDNA by Drs R. J. Robb and B. Freimark (E.I. Du Pont de Nemours & Co. Glenolden Laboratories, Glenolden, PA).

### Isolation and activation of human thymocyte

Thymocytes were isolated from sections of thymus obtained in the course of cardiac surgery of infants and young children as described [19]. The expression of IL-2R $\beta$  was studied on freshly isolated thymocytes and on thymocytes cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin and 0.25  $\mu$ g/ml amphotericin (complete medium), at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Thymocytes were activated as described below.

### Detection of the expression of IL-2R $\beta$ by indirect immunofluorescence

Freshly isolated thymocytes and thymocytes activated with Con A, and Con A in combination with rIL-2 or TPA were incubated in complete media for 24–48 h in flat-bottomed microwells (200  $\mu$ l,  $6 \times 10^6$  cells/ml) as indicated.

Cells were stained with MoAb Mik  $\beta_1$  1/500 and with FITC-anti-IgG2a. The expression of IL-2R $\alpha$  was blocked by the addition at the initiation of cultures of 10  $\mu$ g of 2A3 MoAb, an IgG1 MoAb which is not recognized by anti-IgG2a. Addition of 2A3 MoAb at the time of staining served as control.

### Accumulation of mRNA specific for the $\alpha$ - and the $\beta$ -chain of IL-2R

Total cellular RNA was isolated by a modification of the guanidium method [21]. RNA was extracted with phenol and heat denatured with 2.2 M formaldehyde. Briefly, nucleic acids were isolated by guanidium isothiocyanate lysis, and total cellular RNA was separated by gradient centrifugation in cesium chloride. After extraction with phenol and quantification, RNA was heat denatured in 1 M sodium chloride/50 mM sodium phosphate (pH 7.0)/6% formaldehyde at 60°C for 10 min. Specified amounts of total cellular RNA were blotted

onto 0.45  $\mu$ m nitrocellulose filters (Schleicher & Schuell, Keene, NH) by using a Schleicher & Schuell slot-blot apparatus. Filters were air-dried, baked at 80°C for 2 h, prehybridized for 4 h at 37°C and hybridized for 16 h at 37°C. Hybridizations were performed with the nick-translated IL-2R $\beta$  cDNA probe according to established procedures [22]. Following hybridization, filters were washed under stringent conditions, air-dried, and exposed to X-ray films (Kodak X-Omat 5) intensified by Dupont Cronex Quanta 3 screens at  $-70^\circ\text{C}$ .

Northern blot analysis of IL-2R $\beta$  mRNA was carried out on 1% denaturing agarose gels containing 0.66 M formaldehyde and subsequently transferred to 0.45  $\mu$ m nitrocellulose filters (Schleicher & Schuell) by capillary transfer [23].

## RESULTS

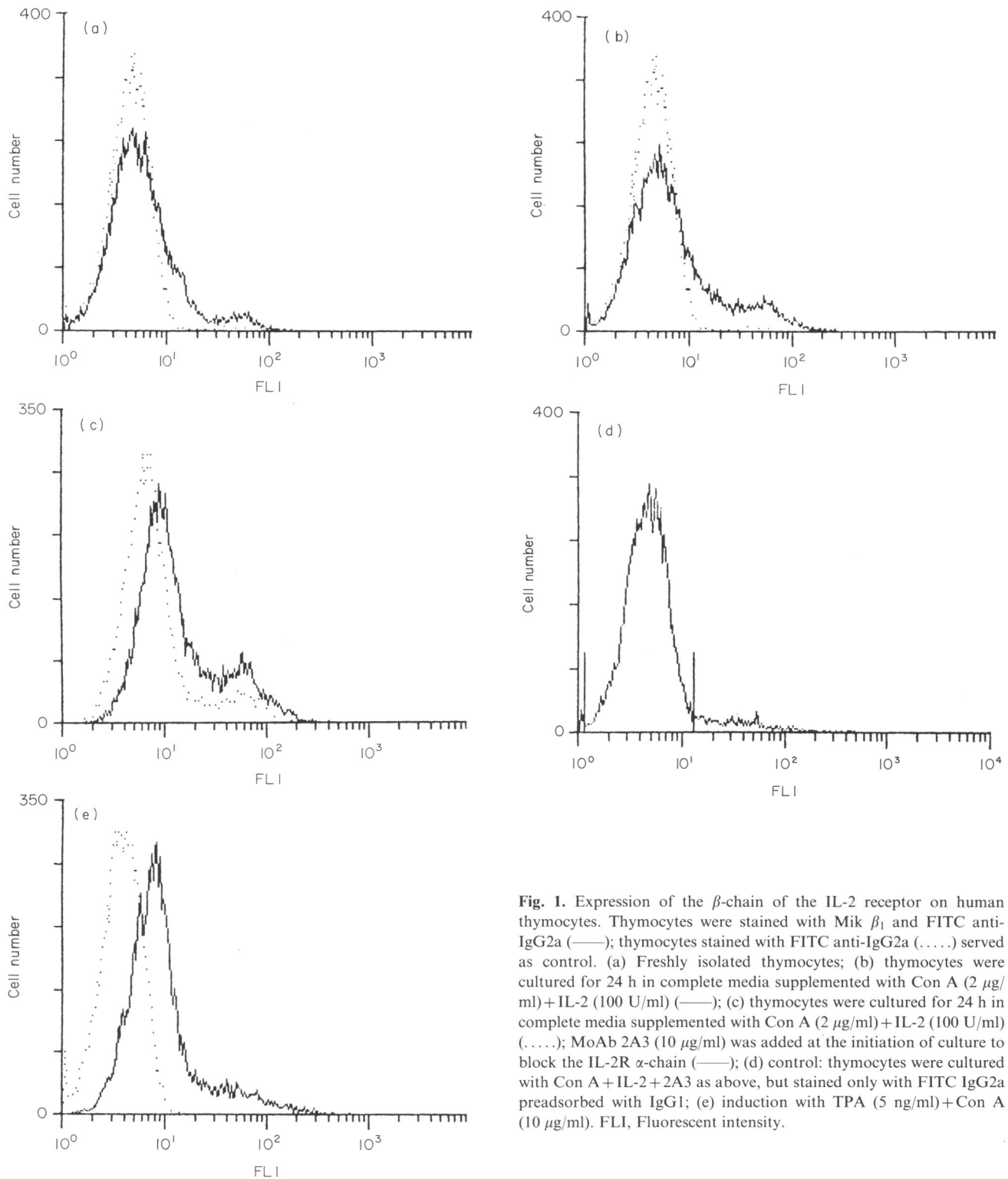
### Expression of IL-2R $\beta$ (p75) subunit on thymocytes

The expression of IL-2R $\beta$  was determined on thymocytes derived from seven children under the age of 3 years who underwent cardiac surgery for congenital defects, but were otherwise in good health. IL-2R $\beta^+$  cells were determined by indirect fluorescence with MoAb Mik  $\beta_1$  and FITC-anti-IgG2a on freshly isolated thymocytes, on thymocytes cultured in complete media, and on thymocytes induced with Con A, Con A + IL-2, Con A + TPA, in the presence and in the absence of CsA. In the majority of samples (4/7) of freshly isolated thymocytes IL-2R $\beta^+$  cells were detected; in the other three specimens constitutive expression of IL-2R $\beta$  was below the limit of detection by indirect immunofluorescence (Table 1). The number of thymocytes expressing IL-2R $\beta^+$  cells ranged from 1% to 14% (Table 1). The constitutive expression of IL-2R $\beta^+$  on freshly isolated thymocytes of specimen 2 is represented in Fig. 1a. The addition of Con A in suboptimal concentrations (2  $\mu$ g/ml) was insufficient to raise the expression of IL-2R $\beta$  as was the addition of IL-2 alone (100 U/ml); however, activation with Con A (2  $\mu$ g/ml) in combination with IL-2 (100 U/ml) increased the number of thymocytes expressing IL-2R $\beta$  from 14% to 21% (Fig. 1b). Thus, the addition of IL-2 to thymocytes suboptimally induced with Con A enhanced the expression of

**Table 1.** Expression of IL-2R $\beta^+$  on freshly isolated thymocytes and thymocytes expressing IL-2R $\beta$

| Specimen | IL-2R $\beta^+$ cells (%) |              | Con A + IL-2<br>+ MoAb 2A3 |
|----------|---------------------------|--------------|----------------------------|
|          | None                      | Con A + IL-2 |                            |
| 2        | 14                        | 21           | 44                         |
| 5        | 10                        | 16           | 29                         |
| 6        | 3                         | 5            | 14                         |
| 1        | 1                         | 3            | ND                         |
| 3        | <1                        | ND           | ND                         |
| 4        | <1                        | ND           | ND                         |
| 7        | <1                        | ND           | ND                         |

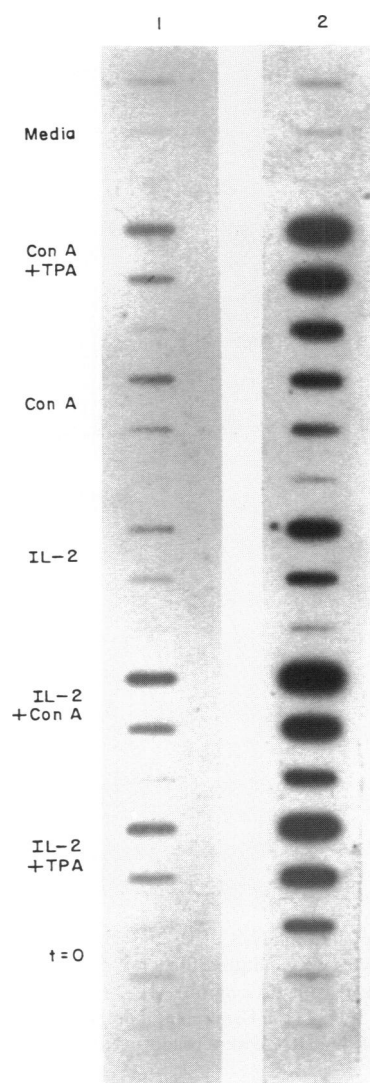
The increase became more apparent when MoAb 2A3 (10 mg/ml), which recognizes the  $\alpha$ -subunit of IL-2R was added to the cultures. Thymocytes were cultured in complete medium for 24 h and stained with Mik  $\beta_1$  and FITC anti-IgG2a as described in Materials and Methods. ND, not done.



**Fig. 1.** Expression of the  $\beta$ -chain of the IL-2 receptor on human thymocytes. Thymocytes were stained with Mik  $\beta_1$  and FITC anti-IgG2a (—); thymocytes stained with FITC anti-IgG2a (....) served as control. (a) Freshly isolated thymocytes; (b) thymocytes were cultured for 24 h in complete media supplemented with Con A (2  $\mu$ g/ml) + IL-2 (100 U/ml) (—); (c) thymocytes were cultured for 24 h in complete media supplemented with Con A (2  $\mu$ g/ml) + IL-2 (100 U/ml) (....); MoAb 2A3 (10  $\mu$ g/ml) was added at the initiation of culture to block the IL-2R  $\alpha$ -chain (—); (d) control: thymocytes were cultured with Con A + IL-2 + 2A3 as above, but stained only with FITC IgG2a preadsorbed with IgG1; (e) induction with TPA (5 ng/ml) + Con A (10  $\mu$ g/ml). FLI, Fluorescent intensity.

IL-2R $\beta$ . Next, the effect on the expression of IL-2R $\beta$  by blocking the  $\alpha$ -subunit of the IL-2 receptor with MoAb 2A3 was examined. As shown in Fig. 1c, the number of thymocytes induced with Con A + IL-2 which expressed IL-2R $\beta^+$  could be further increased to 44% by binding of 2A3 MoAb to the  $\alpha$ -subunit. The blocking of the  $\alpha$ -subunit apparently prevented the joining of the two subunits to form high affinity receptors and thus increased the number of  $\beta$ -subunits that could be detected

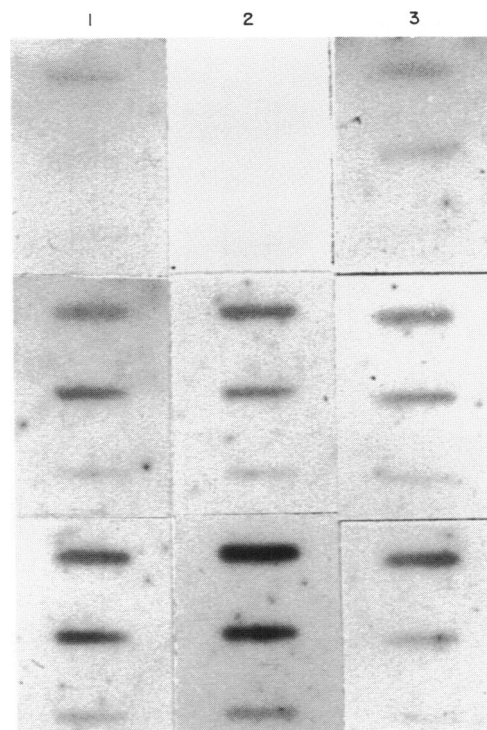
on the cell surface. Thymocytes cultured under the same conditions and stained solely with FITC-anti-IgG2a served as a control for the detection of non-specific fluorescent cells (Fig. 1d). The binding of mouse FITC-IgG2a and the addition of MoAb 2A3 at the time of staining also served as controls (data not shown). The expression of IL-2R $\beta$  could be induced by the addition of TPA + Con A to 32% (Fig. 1e). CsA did not decrease IL-2R $\beta$  expression (data not shown).



**Fig. 2.** Effect of IL-2 on the accumulation of IL-2R $\beta$  and of IL-2R $\alpha$  mRNA. Slot-blot analysis of thymocyte total RNA hybridized to IL-2R $\beta$ -chain cDNA (lane 1) and to IL-2R $\alpha$ -chain cDNA (lane 2). Thymocytes ( $1 \times 10^8$ /ml) were cultured for 48 h under the conditions as indicated. Determination of the accumulation of IL-2R $\beta$ - and IL-2R $\alpha$ -specific mRNA was carried out on aliquots of the same cultures obtained from one representative thymic specimen. Fifteen, 7.5 and 1.5  $\mu$ g of RNA were applied for each experimental condition. Experimental conditions are shown on both lanes (top to bottom): 1. media; 2. Con A (10  $\mu$ g/ml)+TPA (5 ng/ml); 3. Con A (2  $\mu$ g/ml); 4. IL-2 (100 U/ml); 5. IL-2 (100 U/ml)+Con A (2  $\mu$ g/ml); 6. IL-2 (100 U/ml)+TPA (5 ng/ml); 7. freshly isolated thymocytes before incubation ( $t=0$ ).

*Effect of IL-2 and of other inducers on the accumulation of IL-2R $\beta$ - and IL-2R $\alpha$ -specific mRNA in human thymocytes*

Thymic specimens from four children were examined. In freshly isolated thymocytes (Fig. 2, lane 1, bottom,  $t=0$ ), and in thymocytes cultured for 48 h (Figs 2 and 3) and for 6 h (Fig. 3) in unsupplemented complete media (Figs 2–4), a small amount of IL-2R $\beta$ -specific mRNA was detectable. The addition of Con A + TPA, of IL-2 alone, or of Con A in suboptimal concentrations (2  $\mu$ g/ml) resulted in an increase in the accumulation of IL-2R $\beta$  mRNA over that seen in thymocytes cultured in complete media (Figs 2–4). This accumulation was further



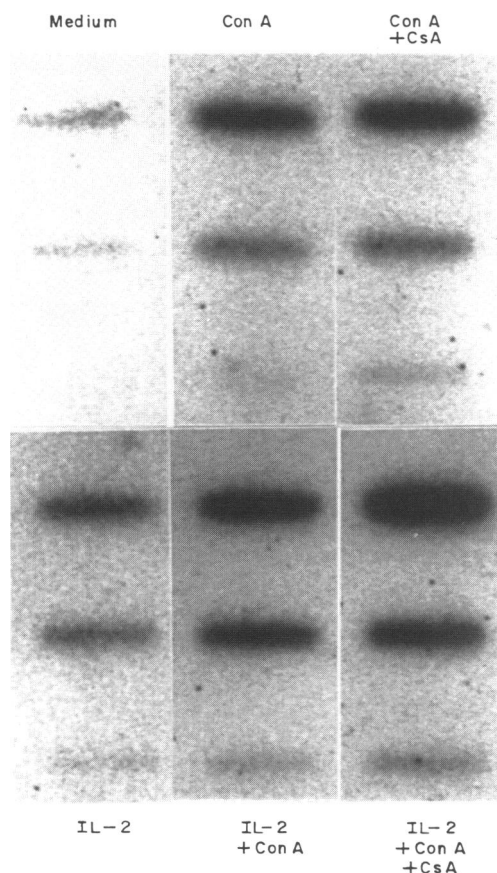
**Fig. 3.** Effect of CsA on the accumulation of IL-2R $\beta$  mRNA of thymocytes induced for 48 h, with Con A or TPA, IL-2, and with Con A + TPA. IL-2R $\beta$  mRNA was induced in activated thymocytes and stimulation with the combination of two inducing agents further enhanced the expression of IL-2R $\beta$  mRNA. CsA did not inhibit mRNA accumulation. Slot-blot analysis was performed as described for Fig. 2. Fifteen, 7.5 and 1.5  $\mu$ g were applied for each experimental condition listed. Lane 1, top to bottom: 1. media; 2. Con A (2  $\mu$ g/ml); 3. Con A (2  $\mu$ g/ml)+IL-2 (100 U/ml); Lane 2: 1. no sample; 2. Con A (2  $\mu$ g/ml)+CsA (1  $\mu$ g/ml); 3. Con A (2  $\mu$ g/ml)+IL-2 (100 U/ml)+CsA (1  $\mu$ g/ml); Lane 3: 1. TPA (5 ng/ml); 2. IL-2 (100 U/ml); 3. TPA (5 ng/ml)+IL-2 (100 U/ml).

enhanced by the addition of IL-2 in combination with Con A (Figs 2–4) or with IL-2 in combination with TPA (Fig. 2 and Fig. 3, lane 3). The effect of these inducers on the  $\alpha$ -subunit of IL-2R in aliquots of the same cultures is shown in Fig. 2, lane 2. The effects are similar; however, the amount of IL-2R $\alpha$  mRNA far exceeded that of IL-2R $\beta$  mRNA. This observation shows that IL-2 by itself induces both IL-2R $\beta$ - and IL-2R $\alpha$ -specific mRNA, and that IL-2 enhances IL-2R $\beta$  mRNA accumulation in thymocytes suboptimally induced with Con A within 6 h. The effect of IL-2 and that of suboptimal concentrations of Con A was more marked following 6 h, at a time when the cell population had as yet not been renewed.

*Effect of CsA on the expression of IL-2R $\beta$  mRNA*

In this experiment thymocytes were induced with Con A (2  $\mu$ g/ml), TPA (5 ng/ml) or IL-2 (100 U/ml) alone, and by a combination of Con A + IL-2, TPA + IL-2, with and without CsA. CsA did not inhibit IL-2R $\beta$  mRNA expression in any of these experimental conditions.

The induction of the accumulation of IL-2 $\beta$  mRNA in thymocytes obtained from a different specimen, cultured for only 6 h, is represented in Fig. 4. IL-2R $\beta$  is induced by IL-2 (lane 1, bottom), by Con A (lane 2, top), by IL-2 + Con A (lane 2,

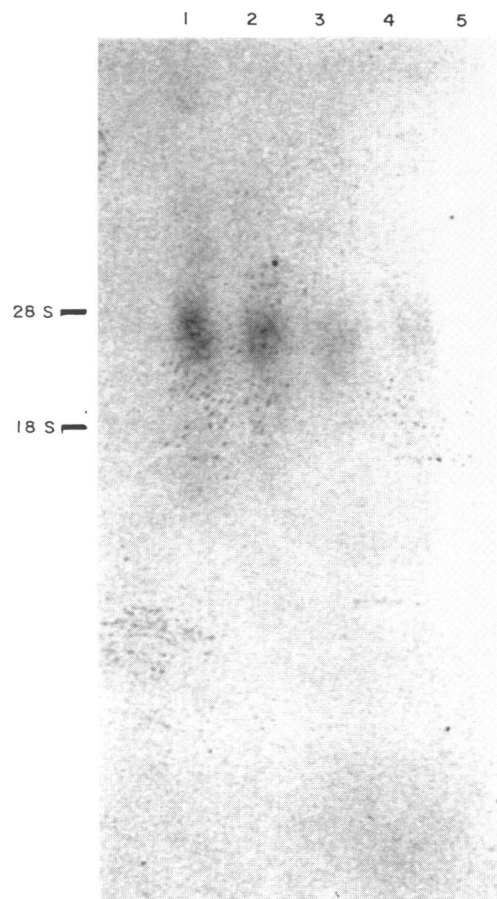


**Fig. 4.** Effect of IL-2 and CsA on the accumulation of IL-2R $\beta$ -specific mRNA of thymocytes incubated for 6 h. Slot-blot analysis of total thymocyte RNA hybridized to IL-2R  $\beta$ -chain cDNA. IL-2 increases the accumulation of IL-2R $\beta$  mRNA of thymocytes induced with Con A and CsA does not inhibit the IL-2R $\beta$  mRNA accumulation. Fifteen, 7.5 and 1.5  $\mu$ g were applied for each experimental condition. Lane 1, top, thymocytes cultured in complete media; bottom, in media supplemented with IL-2 (100 U/ml); lane 2, top, Con A (2  $\mu$ g/ml); bottom, IL-2 (100 U/ml) + Con A (2  $\mu$ g/ml); lane 3, top, Con A (2  $\mu$ g/ml) + CsA (1  $\mu$ g/ml); bottom, IL-2 (100 U/ml) + Con A (2  $\mu$ g/ml) + CsA (1  $\mu$ g/ml), as indicated.

bottom). CsA did not affect mRNA accumulation of thymocytes stimulated with Con A (lane 3, top), or with IL-2 + Con A (lane 3, bottom). The expression of  $\beta_2$ -microglobulin mRNA was not affected (data not shown).

#### *Northern blot analysis of the accumulation of IL-2R $\beta$ mRNA in activated thymocytes*

The induction of IL-2 $\beta$  mRNA was further demonstrated by Northern blot analysis in thymocytes cultured for 6 h (Fig. 5). Thymocytes cultured with Con A + TPA (Fig. 5, lane 1), or with Con A + IL-2 (lane 2), accumulated more IL-2R $\beta$  mRNA than those cultured with Con A (lane 3); or with IL-2 (lane 4) alone. In thymocytes cultured in unsupplemented media (lane 5) mRNA was not detected.



**Fig. 5.** Northern blot analysis of IL-2R $\beta$  mRNA of activated thymocytes. Thymocytes were cultured for 6 h with Con A (2  $\mu$ g/ml) + TPA (5 ng/ml). Lane 1, Con A (2  $\mu$ g/ml) + IL-2 (100 U/ml); lane 2, Con A (2  $\mu$ g/ml); lane 3, IL-2 (100 U/ml); lane 4 and in unsupplemented media lane 5, 15  $\mu$ g of total RNA were used for Northern blotting.

## DISCUSSION

The present study demonstrates that the  $\beta$ -chain of the IL-2 receptor is constitutively expressed on human thymocytes. We detected IL-2R $\beta$  expression by examining the binding of Mik  $\beta_1$ , a MoAb directed against the  $\beta$ -subunit to thymocytes (Fig. 1). The expression of IL-2R $\beta$  can be demonstrated on thymocytes which do not express IL-2R $\alpha$  and IL-2R $\beta$  expression can be increased if thymocytes are activated by Con A in combination with IL-2 or TPA. The increase in IL-2R $\beta$  becomes more apparent with the prevention of the association of the  $\alpha$ -chain with the  $\beta$ -chain, due to the binding of MoAb 2A3 to IL-2R $\alpha$  of activated thymocytes. A similar observation has been made by Tsudo *et al.* [16] in activated T cells.

Other investigators have detected the expression of IL-2R $\beta$  on human thymocytes by studying crosslinking of the surface membrane and the binding of  $^{125}$ I IL-2 [7,9]. By this method the expression of the  $\beta$ -chain in the absence of the  $\alpha$ -chain could be detected even in the presence of an excess of MoAb anti-Tac [9]. The constitutive expression of the  $\beta$ -chain in immature thymic cells was discovered in transgenic mice by showing that functional human IL-2 receptors could be expressed in lymphocytes [24]. However, the expression of functional IL-2R $\beta$  could not be detected in transfected mouse fibroblasts [16].

Earlier studies from our laboratory had shown that human thymocytes can be induced to grow in long term culture even though the  $\alpha$ -chain of IL-2R was initially not detectable and its expression remained low for several days when thymocytes were cultured with IL-2 in the absence of lectins [17]. This observation, and the demonstration that IL-2 upregulated Tac antigen, suggested the presence of an additional receptor which could bind IL-2, induce high affinity receptors and transmit the signal for growth [6,17,19]. A non-Tac peptide which binds IL-2 was demonstrated in the gibbon T cell line MLA 144, in NK cells [4,25] and identified as a 75p peptide in LGL cells [4,26].

The existence of additional subunits of the mouse IL-2R has most recently been reported, adding to the complexity of the structure of this receptor [16,27]. Recently, the generation of MoAbs directed against the  $\beta$ -subunit permitted the direct demonstration by immunofluorescence of the expression of IL-2R $\beta$  on the cell surface of subsets of peripheral T cells and NK cells [12,16].

The cloning of the IL-2R $\beta$  gene enabled us for the first time to demonstrate the modulation of the expression of the accumulation of IL-2R $\beta$ -specific mRNA and we showed that the regulation of the  $\beta$ -subunit was analogous to that of the  $\alpha$ -subunit even within 6 h (Figs 2–5). We provide evidence that IL-2R $\beta$  mRNA was expressed in human thymocytes and that this expression was induced by IL-2, by Con A, by TPA and by IL-2 in combination with Con A or TPA and by Con A + TPA (Figs 2–5). The effect of IL-2 on the induction of IL-2 $\beta$  mRNA within 6 h, at a time when the thymocyte population had as yet not been renewed (Figs 4 and 5), was more marked than that following 48 h. The reasons for this difference are not entirely clear. The observed difference could be due to the rate of IL-2 $\beta$  mRNA turnover; or to a difference in the populations expressing IL-2R $\beta$  at different times. In addition, we demonstrated that the expression of IL-2R $\beta$  was not inhibited by CsA, an immunoregulatory drug which inhibits the activation of T cells and thymocytes and the accumulation of lymphokine-specific mRNA produced by thymocytes [18,28].

In conclusion, thymocytes express IL-2R $\beta$  constitutively, and regulation of the expression of IL-2R $\beta$ -specific mRNA is dependent on the activation of thymocytes by IL-2, by Con A, or by TPA or by a combination of IL-2 + Con A or IL-2 + TPA.

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